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Accumulation of nitrite in denitrifying barriers when phosphate is limiting

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Abstract

Permeable in situ denitrifying barriers can remove nitrate from groundwater. Barriers may be constructed by filling an excavated area with a porous mixture of sand, fine gravel, and substrate or by the injection of a nonaqueous phase substrate into an aquifer. The substrate stimulates the development of a denitrifying microbial community by providing an electron donor. The objective of this study was to determine the ability of denitrifying barriers to function under low-phosphate conditions. Sand columns injected with a soybean oil emulsion were used as laboratory models of denitrifying barriers. When a natural groundwater containing 17 mg l^{-1} nitrate-N and 0.009 mg l^{-1} phosphate-P was pumped through the columns, only a small amount of nitrate was removed from the water and, in some effluent fractions, 52% to 88% of the influent nitrate had converted to nitrite. Nitrite also accumulated when the phosphate concentration of the groundwater was increased to 0.040 or 0.080 mg l^{-1} phosphate-P. Only when a 0.160 mg l^{-1} phosphate-P supplement was added to the groundwater was there a loss of nitrate without a large accumulation of nitrite. The addition of solid calcium phosphate or rock phosphate to the sand columns was found to provide adequate phosphate for denitrification in short-term studies. These studies point out the need to ensure that adequate phosphate is present in denitrifying barriers especially when such barriers are used beneath phosphate-binding soils. Published by Elsevier Science B.V.

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1. Introduction

The presence of large amounts of nitrate in groundwater is detrimental to the health of humans and animals and is damaging to the environment (Goodrich et al., 1991; Bouchard

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et al., 1992; Vitousek et al., 1997; Howarth et al., 2000). While natural sources of nitrate contamination do exist (Edmunds and Gaye, 1997), it is the higher levels of nitrate contamination usually associated with anthropogenic sources that are of the most concern (Foster et al., 1982; Kross et al., 1993; Mueller et al., 1995). Several laboratories have investigated the use of denitrifying barriers, biobarriers, or walls as a method of cleansing nitrate from flowing groundwater (Hunter and Follett, 1995; Robertson and Cherry, 1995; Hunter et al., 1997; Schipper and Vojvodic-Vukovic, 1998; Robertson and Anderson, 1999). These barriers (Fig. 1) are a potentially valuable tool for the remediation of nitrate-contaminated groundwaters and for protecting groundwater from nitrate contamination.

In deeper soils and aquifers, the activity of denitrifying microorganisms is often limited by the availability of an electron donor or energy source (Myrold and Tiedje, 1985; Lalisse-Grundmann et al., 1988; Weier et al., 1992; Starr and Gillham, 1993). Denitrifying barriers function by providing an energy source in a permeable matrix. Barriers may be constructed by back-filling a trench with a mixture of sand, gravel, and substrate (Robertson and Cherry, 1995; Schipper and Vojvodic-Vukovic, 1998; Robertson and Anderson, 1999). It may also be possible to form permeable barriers by injecting a nonaqueous phase substrate, such as a vegetable oil or oil emulsion, into a permeable section of an aquifer (Hunter and Follett, 1995; Hunter et al., 1997; Lee et al., 2000, 2001; Zenker et al., 2000; Hunter, 2001).

Phosphate is an essential microbial nutrient, and supplemental phosphate may be needed when denitrification walls are used to remediate aquifers located beneath soils with high-phosphate retention or other low-phosphate waters. Soils that retain or bind phosphate are common in the western United States (Lewis et al., 1950). Movement of phosphate through such soils is much slower than that of nitrate (Gonzales-Pradas et al.,

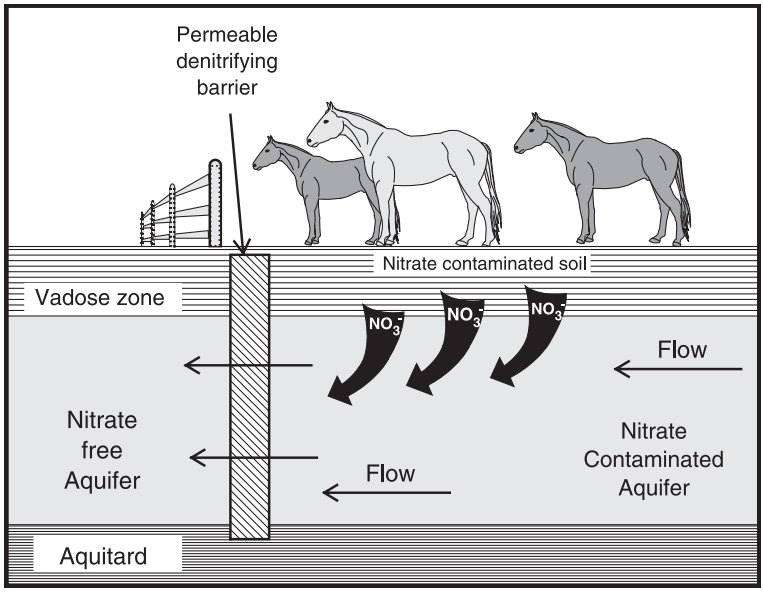

Fig. 1. Hypothetical use of a denitrification wall to protect uncontaminated portions of an aquifer from a contaminated portion of an aquifer.

1993), and low levels of phosphate may limit denitrification in deep soils and underlying aquifers even when the overlying soils are contaminated with high levels of both phosphate and nitrate. This situation might be relatively common beneath livestock pens located on phosphate-binding soils. The objective of this laboratory study was to determine the ability of denitrifying barriers to function under low-phosphate conditions by investigating the denitrifying activity of a laboratory denitrifying barrier supplied with groundwater from beneath a cattle pen located on a phosphate-binding soil.

2. Materials and methods

2.1. Study site

Water samples for this study were collected from a well adjacent to the cattle pens at the Central Plains Experimental Range (CPER). The CPER is located in northeastern Colorado and has a semiarid climate and a shortgrass steppe ecosystem (Shoop et al., 1989; USDA Agricultural Research Service, 1990). The CPER cattle pens see only occasional use through much of the year, but twice a year, in the spring and fall, 900–1000 head of cattle are held in the pens for 1 to 2 days. The pens have been in use for ~ 60 years (Shoop et al., 1989), and this level of usage has been steady since ~ 1988 (Frasier, personal communication). The soil at the cattle pens is a relatively flat (< 3% slope), unstructured Vona sandy loam, a deep, well to excessively drained, mixed, mesic Ustollic Haplargids (USDA Soil Conservation Service, 1982).

Groundwater for this study was collected from a 13.5-m-deep well located 41 m south and downhill from the pens. The water table is about 8.5 m below the soil surface. The groundwater from this well is contaminated with nitrate from the adjacent cattle pens (Hunter, 1998). During this study, water collected from the well was found to contain about 17 mg l^{-1} nitrate-N.

2.2. Denitrifying barriers

Sand columns were used to evaluate the ability of soybean oil to stimulate denitrification with the CPER groundwater. The columns were water-jacketed glass chromatography columns, 2.5 × 30 cm, equipped with flow adapters and nylon screens. The support matrix was 30-grit (0.35-mm sieve size) washed quartz sand packed to a bulk density of 1.49 g cm^{-3}. Columns were maintained in the dark at 15 °C. CPER groundwater held at 4 °C was pumped, in an upward direction, through the columns by a peristaltic pump at a flow rate of about 110–120 ml/day (equivalent to a groundwater flow rate of about 0.51 m/day). Laboratory-scale denitrifying barriers were formed in the columns by injecting a soybean oil emulsion onto the bottom of the column. The emulsion was formed by forcing a mixture of soybean oil (1 ml) and groundwater (9 ml) back and forth through an 18-gauge syringe needle 10 times.

In an initial study, I examined the ability of laboratory denitrifying barriers to remove nitrate from unaltered CPER groundwater. For this study, CPER groundwater was pumped through two sand columns containing denitrifying barriers for ~ 4 weeks and the column

effluents collected and analyzed for nitrate and nitrite three times per week. No phosphate supplements were added to these columns.

In a second study, the ability of laboratory denitrifying barriers to remove nitrate from CPER groundwater containing 0.040, 0.080, or 0.160 mg l^{-1} phosphate-P was examined. Columns, two in each set, received CPER groundwater supplemented with potassium phosphate as indicated. Column effluents were collected and analyzed for phosphate, nitrate, and nitrite three times per week.

In a third study, CPER groundwater was supplemented with sufficient nitrate to produce groundwater containing 16, 32, and 64 mg l^{-1} nitrate-N. Columns, two in each set, received CPER groundwater supplemented with 0.160 mg l^{-1} phosphate-P and no nitrate-N supplement (16 mg l^{-1} nitrate-N water), or supplemented with sufficient nitrate to yield a water containing 32 or 64 mg l^{-1} nitrate-N. The soybean oil emulsion, formed as described above, was added to the column at the end of the first week of operation rather than at the start of the study. Column effluents were collected and analyzed for nitrate and nitrite three times per week.

For the fourth study, the denitrifying barriers in the columns were supplemented with solid soft rock phosphate or calcium phosphate (Biofos™) to evaluate their ability to serve as a source of phosphate for denitrification barriers. At the start of the study, rock phosphate or calcium phosphate was ground to a fine power with a mortar and pestle. This material was blended with the sand that made up the first 5 cm of the influent end of the sand columns. Three sets of columns, two columns in each set, were prepared. The control set received no supplemental phosphate, and the two treatment sets received either rock or calcium phosphate. CPER groundwater containing no supplemental phosphate was pumped through the columns for 10 weeks and the nitrate and nitrite content of the effluent waters were assayed three times per week as described below.

2.3. Nitrate and nitrite assay

The amount of nitrate and nitrite in column effluents was measured using an HPLC equipped with a UV detector operated at 220 nm. Elution buffer was a pH 7.0 buffer containing 1 mM sodium phosphate and 20 mM sodium chloride mixed 4:1 with methanol. A flow of 1.8 ml per min was used. The column was a 4.1 × 100 mm Hamilton PRP-X100 anion exchange column. The assay was modified from Hamilton application #140.

2.4. Phosphate assays

The amount of phosphate in the column effluents and in the CPER groundwater was determined using an ascorbic acid method as described in Standard Methods for the Examination of Water and Wastewater (1992).

2.5. Statistical comparisons

All statistical comparisons presented in the text are standard error of the mean computations and comparisons presented in figures are standard deviation. All comparisons were made using the Instat® computer program (GraphPad Software).

3. Results

3.1. Initial barrier study

Groundwater obtained from the well next to the CPER cattle pens contained ~ 17 mg l^{-1} nitrate-N. When this groundwater from the CPER well was used as a source of water for the laboratory denitrifying columns, nitrate removal was delayed and incomplete. No disappearance of nitrate, as water was pumped through the columns, was observed during the first 15 days of the study (Fig. 2). In contrast, in previous studies where water from a shallow aquifer beneath an agricultural field was used, nitrate was rapidly and completely removed via denitrification from water pumped through similar denitrifying columns (Hunter et al., 1997). After 15 days, the amount of nitrate in the effluent water decreased, but this decrease was accompanied with an increase in the amount of nitrite present in the effluent waters. In the column fractions that accumulated nitrite (fractions 9–13 in Fig. 2), between 52% and 88% of the nitrate-N that was removed from the groundwater was recovered as nitrite-N.

3.2. Phosphate additions

Although the surface soils beneath the cattle pens and above the aquifer contained a considerable amount of phosphate (Hunter, unpublished), the groundwater beneath the pens was found to contain only 0.009 mg l^{-1} phosphate-P. When this groundwater was

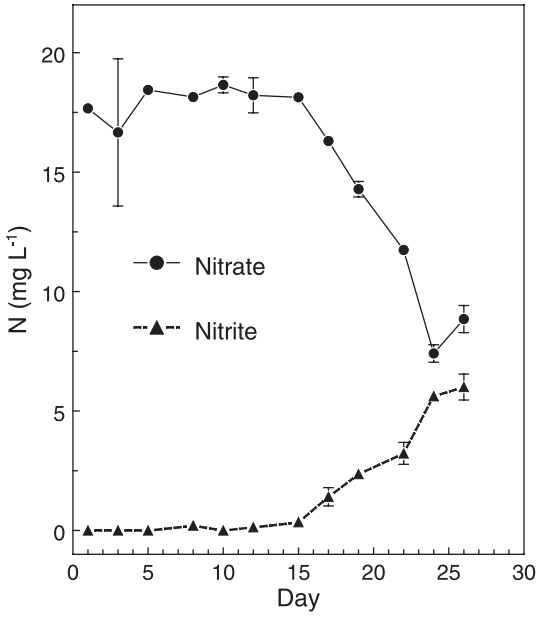

Fig. 2. Presence of nitrite in effluents from laboratory denitrification barriers when no supplemental phosphate is supplied. Each bar is an average of two measurements. Error bars indicate standard deviation.

supplemented with sufficient additional phosphate to give a final concentration of 0.040 mg l^{-1} phosphate-P (N/P = 400), and the water pumped through the laboratory denitrifying barrier, the nitrate levels in the water declined slowly, at a rate of 0.34 ± 0.04 mg nitrate-N l^{-1} day^{-1}, during the initial 36 days of the study (Fig. 3A). Furthermore, during the last 30 days of the study, nitrate was converted to nitrite in large, almost stoichiometric, amounts, and nitrite accumulations averaged 13.2 ± 0.4 mg l^{-1} N during this 30-day period.

The amount of nitrate-N in the denitrifying barrier effluents decreased more rapidly, at a rate of 1.63 ± 0.23 mg l^{-1} day^{-1} over the initial 15 days of the study, when sufficient phosphate was added to the groundwater to give a final concentration of 0.080 mg l^{-1} phosphate-P (N/P = 200) and the groundwater pumped through laboratory denitrifying

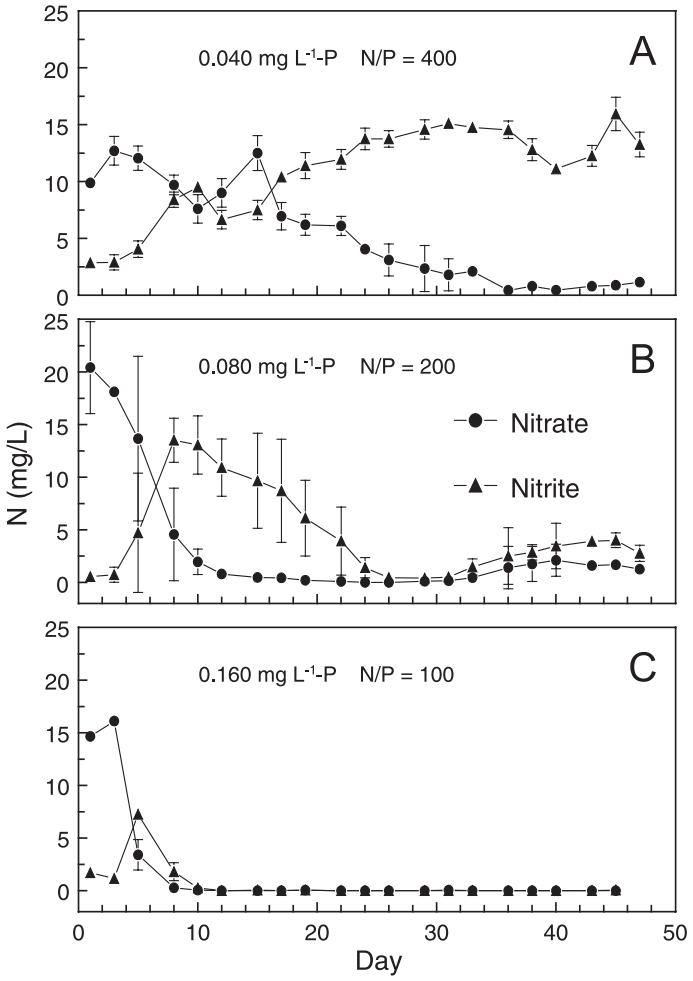

Fig. 3. Effect of supplemental P on nitrite in barrier effluents. Each bar is an average of two measurements. Error bars indicate standard deviation.

barriers. However, transient accumulations of large amounts (>5 mg l^{-1}) of nitrite-N that lasted several weeks were observed at this phosphate level (Fig. 3B).

Increasing the amount of phosphate in the influent groundwater to 0.160 mg l^{-1} phosphate-P (N/P = 100) resulted in a decrease in effluent nitrate-N levels of 2.4 ± 0.8 mg l^{-1} day^{-1} over an 8-day period—a much more rapid decrease than had been observed at the 0.040 or 0.080 mg l^{-1} phosphate-P levels. Also, though briefly exceeding the EPA MCL, the amount of nitrite that was present in the effluents was much less than had been observed when less phosphate was present in the barrier influent water (Fig. 3C). Both nitrate and nitrite declined to undetectable levels during the final weeks of the study.

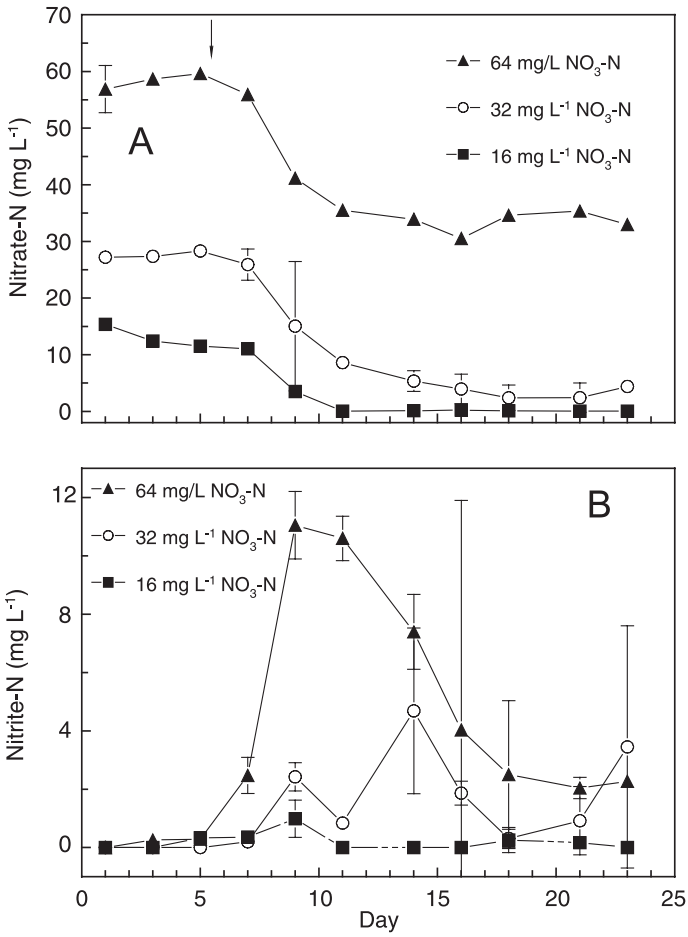

Fig. 4. Change in nitrate (A) and nitrite (B) in column effluents after the addition of the soybean oil emulsion (arrow) to the influent groundwater. Influent waters were supplemented with 0.160 mg l^{-1} phosphate-P and sufficient nitrate to yield a final concentration of 16, 32, or 64 mg l^{-1} nitrate-N. Each data point is an average of two readings. Error bars indicate standard deviation.

The amount of phosphate in the effluents of columns supplied with 0.040, 0.080, and 0.160 mg l^{-1} phosphate-P was monitored during the phosphate addition studies. Effluents from columns supplied with CPER groundwater containing 0.040 or 0.080 mg l^{-1} phosphate-P remained constantly low through the study and averaged 0.0061 ± 0.0011 and 0.0062 ± 0.0009 mg l^{-1} P, respectively. Effluents from columns supplied with groundwater containing 0.160 mg l^{-1} phosphate-P averaged 0.066 ± 0.004 mg l^{-1} P during the first 2 weeks of the study, but these levels declined to 0.012 ± 0.002 mg l^{-1} P by the third and fourth weeks of the study. The low level of phosphate in the effluent fractions of the 0.040 or 0.080 mg l^{-1} phosphate-P columns suggests that microbial nitrate removal activity may have been limited by phosphate availability.

At higher nitrate concentrations, 0.160 mg l^{-1} phosphate-P did not provide adequate amounts of phosphate for the complete removal of nitrate, and accumulations of nitrite were observed. When the amount of nitrate supplied in the influent water was increased to 32 or 64 mg l^{-1} (a 200 and a 400 N/P ratio) and 0.160 mg l^{-1} phosphate-P supplied only about 24 mg l^{-1}, nitrate-N was removed as the water passed through the denitrification barriers in the sand columns (Fig. 4A). Transient accumulations of nitrite occurred with the

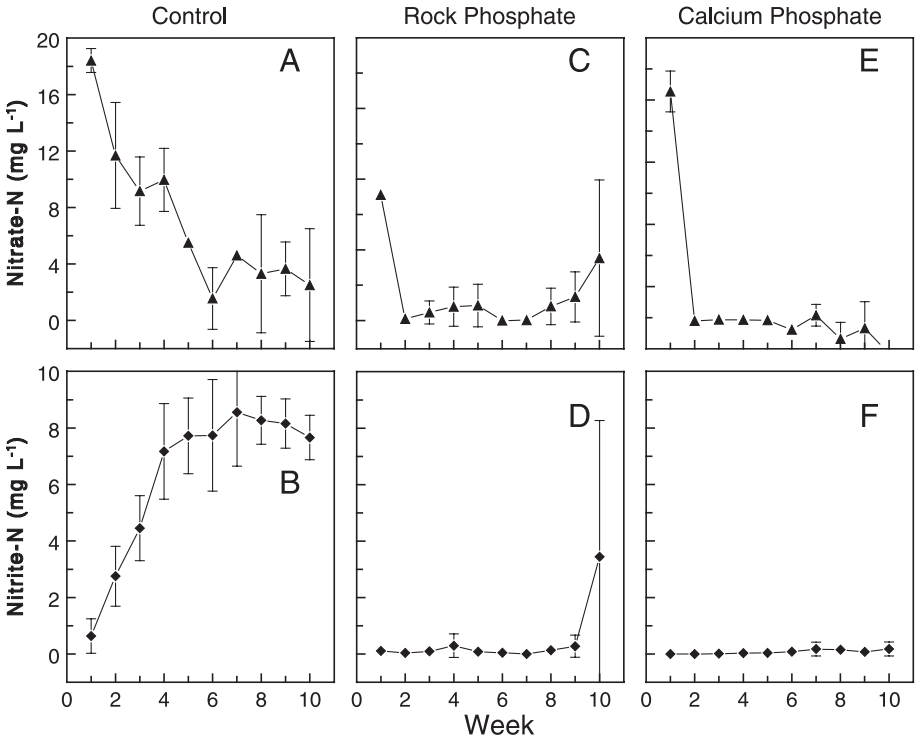

Fig. 5. Effluent nitrate and nitrite content from laboratory-scale barriers that received no phosphate (A and B), solid rock phosphate (C and D), or calcium phosphate (E and F). Each data point is an average of two readings. Error bars indicate standard deviation.

greater accumulation taking place in the columns that were supplied with the larger amount of nitrate in the influent water (Fig. 4B). Nitrite accumulations in the columns supplied with 16 mg l^{-1} nitrate-N, CPER groundwater containing no nitrate supplement (a 100 N/P ratio), did not exceed the EPA standard of 1 mg l^{-1} nitrite-N in this study. This study indicates, as was observed earlier, that an N/P ratio of 100 or less is required to prevent nitrite accumulations in the column effluents.

The addition of solid rock phosphate or calcium phosphate to the laboratory denitrifying barriers at the time the columns were packed provided adequate phosphate for the removal of nitrate without the accumulation of nitrite over the course of a 10-week study (Fig. 5). Nitrate levels in effluents from control columns, which received neither rock phosphate nor calcium phosphate, averaged 5 ± 1.1 mg l^{-1} nitrate-N during the last 8 weeks of the study, while those columns that received rock phosphate averaged 1.2 ± 0.5 and those that received calcium phosphate contained no nitrate. Nitrite levels in the effluents from the control columns averaged 7.5 mg l^{-1} nitrite-N during the last 8 weeks of the study, while the columns that received rock phosphate and calcium phosphate averaged 0.5 ± 0.4 and 0.09 ± 0.02 mg l^{-1} nitrite-N, respectively, over this same time period. Phosphate in column effluents averaged 4.11 ± 0.82, 0.17 ± 0.05, and 0.016 ± 0.008 mg l^{-1} P for the rock phosphate, calcium phosphate, and control columns, respectively, over the course of the 10-week study.

4. Discussion

It would be expected, since the CPER pens were used for livestock and livestock manures are a good source of both phosphorus and nitrogen, that the surface soils from the CPER pens would be high in both phosphate and nitrate. During the $\sim$ 60 years that the cattle pens at the CPER have been in use, nitrate from these surface soils has migrated into the groundwater beneath the pens while phosphate has not. The result is that the groundwaters exceed the EPA drinking water standard for nitrate-N but are low in phosphate. Previous studies have demonstrated that denitrifying barriers formed by the injection of vegetable oil onto sand columns can remove nitrate from flowing water by denitrification (Hunter et al., 1997). However, in this study, the levels of phosphate present in the CPER groundwater were found to be too low for the biological removal of nitrate by the column denitrification barriers. Of most concern, however, was the high levels of nitrite that formed when water from beneath the pens was supplied to the denitrification barriers.

The conversion of nitrate to nitrite by denitrification barriers is highly undesirable as nitrite is more toxic than nitrate. The conversion would present concern in an in situ remediation project, as the EPA maximum contaminant level (MCL) for nitrate-N is 10 ppm while the MCL for nitrite-N is only 1 mg l^{-1} (USEPA, 2001). Thus, in terms of the MCLs, the water that was not supplemented with phosphate from beneath the cattle pens was in a poorer condition after passage through the column denitrification barrier than before. Before passage through the barrier, the water contained 17 mg l^{-1} nitrate-N and the EPA nitrate standard for nitrate was exceeded by 1.7-fold, and, after treatment, nitrite was present at up to 6 mg l^{-1}-N, exceeding the EPA nitrite standard by a factor of 6 (Fig. 3).

Increasing the amount of phosphate in the water to 0.160 mg l^{-1} was found to greatly reduce the accumulation of nitrite. The results demonstrate the importance of assuring that adequate phosphate is present in denitrification barriers.

A review of the literature found no previous reports of nitrite accumulations from the reduction of nitrate when phosphate was limiting. However, factors that influence the growth rate of microorganisms have been shown to cause the accumulation of nitrite by denitrifying microorganisms. Blaszczyk (1993) observed that nitrite accumulations by *Paracoccus denitrificans* correlated with growth rate as controlled by the nutrient content of the growth medium. When *P. denitrificans* was grown in a nutrient-poor medium, growth was slow and a large amount of nitrite accumulated, but when *P. denitrificans* was grown in a rich medium, the growth rate was fast and a lesser amount of nitrite accumulated. Blaszczyk (1993) suggests that the cause for the accumulation of nitrite in minimum media is due to a delayed synthesis of nitrite reductase relative to nitrate reductase. A number of additional factors have also been reported to cause nitrite to accumulate under denitrifying conditions. These include the following: (i) differential repression of nitrate and nitrite reductase synthesis and activity (Korner and Zumft, 1989; Coyne and Tiedje, 1990); (ii) the electron donor present (Blaszczyk, 1993; van Rijn et al., 1996); (iii) competition by nitrate and nitrite reductases for electron donors (Thomsen et al., 1994; van Rijn et al., 1996); iv) the presence of fermentable carbon sources that select for nitrate-respiring bacteria (Wilderer et al., 1987); (v) changes in the pH of the growth media (Glass and Silverstein, 1998); (vi) differences in the maximum rates of reduction by nitrate and nitrite reductases (Betlach and Tiedje, 1981); and (vii) increases in the electrical conductivity of the growth medium (Smith and Doran, 1996). Toxic compounds may also influence the accumulations of nitrite during denitrification. Nitrite and nitrous oxide have been shown to accumulate when denitrification was inhibited by the presence of heavy metals, pesticides, or pesticide derivatives (Mitsui et al., 1964; Bollag and Henneringer, 1976; Bollag and Barabasz, 1979; Bollag and Kurek, 1980).

Blaszczyk (1992, 1993) reported that different microorganisms show different patterns of nitrite accumulation, and the accumulation of nitrite is strongly influenced by the microbial species present. Under similar growth conditions, *P. denitrificans* did not accumulate nitrite, *Pseudomonas stutzeri* first completely transformed nitrate to nitrite and then reduced nitrite to nitrogen gas, while *Pseudomonas aeruginosa* reduced nitrate with transient accumulations of nitrite.

The reason that nitrite accumulated in the column denitrification barriers used in this study under low-phosphate levels was not investigated. One might speculate that an initial reduction of nitrate was favored when phosphate limited growth and that nitrite was only utilized when the electron acceptor needs of the denitrifying population were not met by the amount of nitrate that was available. Alternatively, the accumulation of nitrite might be due to changes in the microbial population over the course of the study.

Rock phosphate or calcium phosphate might prove to be an adequate phosphate source for in situ remediation projects, but long-term studies would need to be conducted to confirm the results observed in the present short-term study. While many factors influence the life of a permeable barrier, it is estimated that denitrification barriers may last many years before the initially applied carbon substrate is exhausted (Blowes et al., 2000; Robertson et al., 2000; Schipper and Vojvodic-Vukovic, 2001). Blowes et al.

(1994) estimated that a denitrifying reactor that contained 5% carbon (as cellulose) by weight might last for decades without the need for additional substrate, while Robertson and Cherry (1995) estimated that an in situ denitrification barrier that contained 2% carbon (as sawdust) should last 20 years or more. If in situ barriers are to be of use in areas with phosphate-poor groundwater, then a long-lasting phosphate source must also be provided. Care would need to be taken to ensure that the phosphate source used does not leach undesirable levels of phosphate into the groundwater downstream of the barrier.

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